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Effect of Carbon Dioxide on Growth of Meat Spoilage Bacteria

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The ability of CO_2 to inhibit respiration and growth of representative strains of seven species of meat spoilage bacteria was examined. *Enterobacter* and *Microbacterium thermosphactum* were unaffected by CO_2. Both respiration and growth of the other species were inhibited. With four of the species (fluorescent and nonfluorescent *Pseudomonas*, *Alteromonas putrefaciens*, and *Yersinia enterocolitica*), the inhibition pattern in a complex medium was similar, and inhibition was incomplete and reached a maximum level at comparatively low concentrations of CO_2. With *Acinetobacter*, inhibition continued to increase with increasing CO_2 concentration. The degree of inhibition with a constant concentration of CO_2 in solution increased with decreasing temperature for all CO_2-susceptible species except the nonfluorescent *Pseudomonas*. Anaerobic growth of CO_2-susceptible facultative anaerobes was unaffected by CO_2.

It has long been known that carbon dioxide inhibits growth of meat spoilage organisms and can be used to extend the shelf life of chilled meat (8). A number of studies have confirmed that significant extension of the storage life of chilled meat can be obtained with atmospheres containing 10 to 20% CO_2, but the detailed results are somewhat conflicting (1, 7, 9). These apparent differences may have arisen because of the paucity of data on the effects of CO_2 on individual bacterial species. An initial examination of the effect of CO_2 on a strain of *Pseudomonas fluorescens* (4) showed that the degree of response to CO_2 differed in minimal and complex medium; in minimal medium the degree of inhibition was proportional to the CO_2 concentration, but in complex medium a maximum degree of inhibition was attained at relatively low CO_2 concentrations. Decrease in the growth temperature increased the degree of inhibition at any CO_2 concentration. There is no clear indication of the extent to which these results can be applied to other spoilage organisms, so the responses to CO_2 of representative strains of common meat spoilage organisms were examined.

MATERIALS AND METHODS

Organisms. Species of *Enterobacter*, fluorescent and nonfluorescent *Pseudomonas*, *Acinetobacter*, *Microbacterium thermosphactum*, *Alteromonas putrefaciens*, and *Yersinia enterocolitica* were used. All bacteria were isolated from spoiled meat and maintained on nutrient agar slopes. Both *Pseudomonas* species and *Acinetobacter* are strict aerobes; all other species are facultative anaerobes.

Oxygen consumption. Cultures were grown at 30°C on a simple salts-glucose medium (4), except in the case of *M. thermosphactum* when yeast extract (0.5 g/liter) was added. Bacteria were pelleted by centrifugation, washed twice, and suspended in 0.1 M phosphate buffer, pH 7.0. Respiration of suspended bacteria was measured in an oxygen electrode cell filled with 10 mM phosphate buffer or with CO_2-bicarbonate buffer in equilibrium with a gas phase of 80% CO_2–20% O_2 diluted with phosphate buffer when filling the cell to give the required CO_2 concentrations. Both buffers were at pH 7.0. Bacterial suspensions (0.05 ml) and substrate solutions (0.1 M; 0.1 ml) were injected into the sealed cell as required.

Growth in liquid medium. All organisms were grown in a magnetically stirred vessel in a simple salts-glucose medium supplemented with yeast extract (4) under atmospheres of air, air/CO_2, (4:1, vol/vol), or oxygen/CO_2 (1:4, vol/vol). Facultative anaerobes were also grown under nitrogen or nitrogen/CO_2 (1:1, vol/vol) atmospheres. The medium was allowed to equilibrate with atmospheres containing CO_2, and the pH was adjusted to 7.0 before inoculation. Growth was measured by the increase in optical density at 550 nm.

Growth on meat. The upper surfaces of 2-cm cubes of meat were inoculated with log-phase cultures of individual species and placed in desiccators (29-cm diameter, 14 meat cubes per desiccator), the bases of which contained 100 ml of water to maintain a saturated atmosphere. The desiccators were filled with air or a mixture of 80% air–20% CO_2 and held in refrigerated cabinets at 3 ± 0.5°C. Meat of high ultimate pH (>6.2) was used as growth of some species (*Acinetobacter*, *A. putrefaciens*, *Y. enterocolitca*) is inhibited when the pH is below 6.0 (2, 3). Slices were removed daily and stomached with 10 ml of peptone water, and suitably diluted samples were spread on nutrient agar plates for estimation of cell densities.

RESULTS

The rates of respiration of both species of *Pseudomonas*, *Acinetobacter*, *Y. enterocolitica*,

and *A. putrefaciens* were reduced in the presence of CO_2. In buffer containing glucose and yeast extract, the minimum rates of respiration were about 60% of the uninhibited rates, except in the case of *Acinetobacter*, where with the maximum CO_2 concentration respiration fell to 25% of normal without apparently reaching a minimum value (Fig. 1). Results were similar with individual substrates (glucose, pyruvate, and serine), but there was some variation of the maximum degree of inhibition with the substrate used. CO_2 had no effect on the respiration of *Enterobacter* or *M. thermosphactum*.

Decreasing the temperature did not change the degree of inhibition by CO_2 of respiration in the nonfluorescent *Pseudomonas*, but the four other CO_2-affected species showed a greater degree of inhibition at low than at high temperatures with a constant CO_2 concentration (Table 1).

Growth in liquid media confirmed that aerobic growth of *Enterobacter* and *M. thermosphactum* was unaffected by CO_2, whereas *Acinetobacter* showed greater susceptibility to high concentrations of CO_2 than the other CO_2-inhibited species (Table 2). The anaerobic growth rates of all facultative anaerobes were unaffected by the presence of CO_2.

When growing on meat at 3°C in an atmosphere of 20% CO_2–80% air, the growth rates of *Enterobacter* and *M. thermosphactum* were the same as in air. The nonfluorescent *Pseudomonas* was the least inhibited of the CO_2-susceptible species, and *A. putrefaciens* was the most strongly inhibited. Growth of the three other species was inhibited to a similar degree (Table 3).

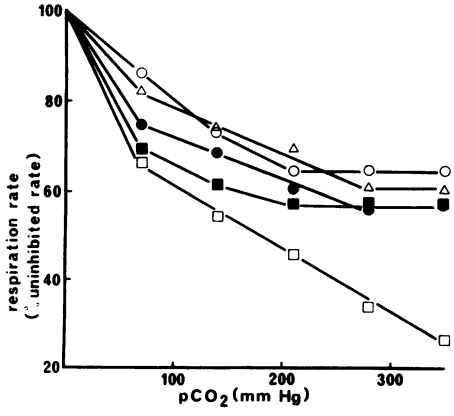

FIG. 1. *Effect of CO_2 on respiration at 30°C of spoilage bacteria in buffer containing glucose and yeast extract. Nonfluorescent Pseudomonas* (○), *fluorescent Pseudomonas* (●), *Acinetobacter* (□), *Y. enterocolitica* (■), *and A. putrefaciens* (△). *Uninhibited respiration rates are given in Table 1.*

TABLE 1. *Effect of temperature on inhibition of bacterial respiration by CO_2 at 140 mmHg (ca. 18,662 Pa) partial pressure in solution*

Organism	Respiration			
	Uninhibited rate (nmol of O_2 min^{-1} mg^{-1})		Inhibited rate (% uninhibited rate)	
	30°C	10°C	30°C	10°C
Nonfluorescent *Pseudomonas*	160	54	72	73
Fluorescent *Pseudomonas*	187	48	74	63
Acinetobacter	118	37	63	44
Y. enterocolitica	94	21	73	58
A. putrefaciens	162	43	79	50

TABLE 2. *Inhibition by CO_2 of aerobic growth at 30°C in minimal medium supplemented with yeast extract*

Organism	Generation time (h^{-1}) with the following % CO_2 in atmosphere:		
	0	20	80
Nonfluorescent *Pseudomonas*	0.9	1.2	1.4
Fluorescent *Pseudomonas*	1.0	1.5	1.7
Acinetobacter	1.1	1.4	2.8
Y. enterocolitica	1.2	1.4	1.7
A. putrefaciens	1.2	1.4	1.5

TABLE 3. *Growth rates of spoilage bacteria on high-pH, dark, firm, dry meat at 3°C in air and in an air–20% CO_2 atmosphere*

Organism	GT (h^{-1})[a]		
	Air	20% CO_2	GT (CO_2)/GT (air)
Nonfluorescent *Pseudomonas*	7.1	8.5	1.20
Fluorescent *Pseudomonas*	7.8	10.0	1.28
Acinetobacter	9.6	12.4	1.29
Y. enterocolitica	11.5	14.7	1.28
A. putrefaciens	9.1	13.6	1.49
Enterobacter	10.8	10.8	1.00
M. thermosphactum	12.1	12.1	1.00

[a] GT, Generation time.

DISCUSSION

In the only previous study on the effect of CO_2 on individual meat spoilage organisms, the authors concluded that gram-negative organisms were more susceptible to inhibition by CO_2 than were gram-positive organisms (10). This is an over-simplification of the situation, because spe-

cies from both groups can be completely insensitive to CO_2. However, the most important gram-positive groups are facultative or strict anaerobes, and anaerobic growth is apparently not inhibited by CO_2, whereas the most important gram-negative organisms are strict aerobes and are susceptible to CO_2 inhibition.

Most CO_2-sensitive species were inhibited when growing in a complex medium in a manner similar to that observed with *P. fluorescens*, in which a maximum degree of inhibition was attained at relatively low CO_2 concentrations and the degree of inhibition at any CO_2 concentration increased with decreasing temperature (4). There are, however, exceptions to both these generalizations.

It is not advisable to use atmospheres containing CO_2 in excess of 20% for storage of meat, because at higher concentrations the appearance of the meat is adversely affected (5, 9). When meat is stored in aerobic atmospheres containing CO_2 at about this level spoilage is delayed, but there is no significant change in the composition of the flora, which is still dominated by pseudomonads (1, 8). This is not surprising because, although *Enterobacter* and *M. thermosphactum* are unaffected by CO_2, at chill temperatures the CO_2-inhibited growth rates of pseudomonads still exceed those of the former species. The species that only assume importance on dark, firm, dry meat, where the high ultimate pH allows their growth, are affected by CO_2 in a similar manner to the pseudomonads, so they would not be advantaged by storage in aerobic atmospheres containing CO_2. The dominant position of pseudomonads in the spoilage flora will therefore be maintained although closer examination may reveal some enrichment in the presence of CO_2 for unaffected species and for the least affected strains of CO_2-sensitive species, such as the nonfluorescent *Pseudomonas*.

The presence of CO_2 will result in a reduction of the aerobic growth rate of a meat spoilage flora of about 25 to 30%, which would give only a similar percentage increase in the storage life. However, a doubling of the storage life can apparently be achieved if CO_2 is applied before growth commences as this produces an extended lag phase in addition to the reduced growth rate of the spoilage flora (1). Some differences in the reported effects of CO_2 on inhibition of aerobic spoilage probably result from differences in the growth phase of the bacteria at the time of CO_2 application.

The basis of CO_2 inhibition of microbial growth has not been elucidated, though direct inhibition of specific enzymes may be involved. The observation that respiration as well as growth is inhibited, whereas anaerobic growth of CO_2-susceptible facultative anaerobes in unaffected, suggests that enzymes of oxidative metabolism may be involved. The possibility that inhibition is the result of a mass action effect by CO_2 on decarboxylating enzymes (6) is unlikely, because in most cases maximum inhibition is not total and occurs at comparatively low CO_2 concentrations, whereas the postulated mechanism should result in a decrease in growth rate directly proportional to CO_2 concentration, and complete inhibition by CO_2 should be possible. The effects of CO_2 on the activities of key enzymes of oxidative metabolism from a CO_2-susceptible and a nonsusceptible species are presently being compared to try to determine the mode of action of CO_2 in inhibiting aerobic growth and respiration of bacteria.

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